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Legionellae isolated from clinical and environmental samples in Spain (1983–90): monoclonal typing of *Legionella pneumophila* serogroup 1 isolates

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SUMMARY

Legionella isolates recovered in 21 different Spanish provinces over 8 years from both clinical (67 isolates) and environmental (181) samples, mostly from case-associated buildings, are described: 92·5% of clinical isolates were *L. pneumophila* serogroup 1 (SG1), only five isolates belonging to other species or serogroups: two *L. pneumophila* SG6, two SG8 and one *L. bozemanii* SG1 not clearly related with clinical infection.

L. pneumophila SG1 accounted for 53·6% of isolates from the environment, followed by SG8 (27·6%), SG3 (9·4%) and SG6 (7·2%). Three isolates were labelled as SG8/10.

Subtyping of *L. pneumophila* SG1 by the standardized panel of monoclonal antibodies revealed 90·3% of clinical and 78·3% of environmental isolates as belonging to Pontiac subgroup. Pontiac isolates were further divided into 55·3% Philadelphia 1 or Allentown 1, 21·9% Benidorm 030E and 20·4% Knoxville 1.

Characterization of samples from four outbreaks in which both clinical and environmental isolates had been recovered permitted the recognition of three Philadelphia 1 or Allentown 1 and one Knoxville 1 strains as the aetiological agents.

INTRODUCTION

During the last decade more than 20 species of *Legionella* and 14 serogroups of *L. pneumophila* have been described. These are encountered in both natural aquatic environments [1], such as lakes and rivers, and in man-made water systems [2, 3], such as pipe systems for cold and hot water and air conditioning installations and have been associated with human infection [4].

L. pneumophila SG1 is the most common isolate both in human samples [4] and environmental sources [3], not always related to clinical cases. In order to recognize these isolates for epidemiological purposes, monoclonal antibody subtyping panels have been developed [5, 6] permitting the separation of isolates into subgroups. Watkins and coworkers [6] recognized three major subgroups named after their type strain: Pontiac, Olda and Bellingham, and reported that

subgroup Pontiac is most frequently associated with the production of infection in the form of outbreaks of legionellosis and may be found in case-associated buildings, whereas the Olda subgroup usually colonizes water systems in non-case-associated buildings and Bellingham can be found both in disease-associated piped water systems and in disease-unassociated ones.

As a consequence of an international collaborative study, a standardized panel of monoclonal antibodies [7] was described, which recognized the three subgroups mentioned above and ten minor subgroups, each with a representative type strain [7, 8].

In this article we describe the species and serogroups of legionellae, received in our laboratory or recovered by us over the past 8 years. Furthermore, the results of subtyping *L. pneumophila* SG1, by using monoclonal antibodies, are included.

MATERIALS AND METHODS

The isolates consisted of 248 legionellae recovered between 1983 and 1990 in different provinces of Spain and sent to us for identification, or isolated in our laboratory (Table 1). Sixty-seven had been isolated from clinical samples and 181 had an environmental origin, frequently from buildings associated with clinical cases.

Isolates were identified as legionellae by positive growth in Buffered Charcoal Yeast Extract medium containing α-ketoglutarate (BCYEα) [9] and absence of growth on blood agar or in BCYEα without cysteine. Species identification was performed by indirect immunofluorescence with rabbit antisera produced in our laboratory [10] to the following species and serogroups: *L. pneumophila* SG1–11, *L. micdadei*, *L. bozemanii* SG1 and SG2, *L. gormanii*, *L. dumoffii*, *L. jordanis*, *L. longbeacheae* SG1 and SG2, *L. wadsworthii* and *L. oakridgensis*. Fluorescein labelled goat antirabbit globulin (DAKO) was used as the conjugate. Antisera to legionellae presenting serological cross-reactions (i.e. *L. pneumophila* SG4, SG5, SG8 and SG10) were highly absorbed with the corresponding type strain to avoid such cross reactivity.

We used the monoclonal antibodies included in the standardized panel [7]. MAB1, MAB2 and MAB3 were obtained from ATCC, 33G2 and 144C2 were kindly provided by Dr Joly (Quebec, Canada) and W268, with the same reactivity as W32, provided by Dr Selkon (Oxford). Typing was carried out by indirect immunofluorescence with formolized antigens and fluorescein labelled antimouse globulin (DAKO). As MAB 32A12 was not available, we were unable to distinguish between Philadelphia 1 or Allentown 1 subtypes and between Oxford 4032E and Camperdown 1 subtypes.

RESULTS

L. pneumophila SG1 accounted for 64·1% of the isolates (Tables 1 and 2), being more frequent in clinical samples (92·5%) than in environmental ones (53·6%). Other legionellae recovered from clinical samples were *L. pneumophila* SG6 and SG8 and *L. bozemanii* SG1 that was isolated from a patient catheter, without clear evidence of producing infection.

Table 1. *Origin of isolates: species and serogroup distribution*

Province	Number of isolates: Total	Clinical	Environmental	Species and serogroup
Albacete	2	2		L. pn 1
Alicante	37		13	L. pn 1
			1	L. pn 8/10
			2	L. pn 6
			21	L. pn 8
Almería	1		1	L. pn 8
Asturias	1		1	L. pn 1
Barcelona	51	31	5	L. pn 1
		2		L. pn 6
		2	8	L. pn 8
			2	L. pn 8/10
		1		L. boz 1
Baleares	43		15	L. pn 1
			2	L. pn 3
			7	L. pn 6
			19	L. pn 8
Bilbao	2	2		L. pn 1
Burgos	1	1		L. pn 1
Canarias	2		1	L. pn 1
			1	L. pn 7
Guadalajara	10		9	L. pn 1
			1	L. pn 8
Madrid	5	5		L. pn 1
Málaga	2		1	L. pn 1
			1	L. pn 6
Navarra	2	2		L. pn 1
Pontevedra	1	1		L. pn 1
Salamanca	3	2	1	L. pn 1
Santander	10		7	L. pn 1
			1	L. pn 3
			2	L. pn 6
Sevilla	1		1	L. pn 6
Tarragona	1	1		L. pn 1
Valencia	17	1	16	L. pn 1
Vizcaya	1	1		L. pn 1
Zaragoza	55	13	28	L. pn 1
			14	L. pn 3
Total	248	67	181	

Samples from the environment yielded a wider variety of legionellae: *L. pneumophila* SG8 followed SG1 in order of frequency (27·6%), followed by SG3 (9·4%) and SG6 (7·2%). Three isolates were labelled as SG8/10.

Subtyping of 159 *L. pneumophila* SG1 isolates (Table 3) revealed 83% as belonging to the Pontiac subtype, being more frequent (90·3%) in clinical samples than in environmental ones (78·3%). Non-Pontiac isolates were more common in environmental (21·5%) than in clinical (9·6%) isolates.

Pontiac isolates were subdivided as follows: 55·3% Philadelphia 1 or Allentown 1, 21·9% Benidorm 030E, 20·4% Knoxville 1, and 2·2% France 5811.

Some subtypes were associated with certain localities: in Zaragoza, 37 of 41 SG1

Table 2. *Distribution in species and serogroups of 248 isolates of legionella*

Species and serogroup	*Number (%)*	*Clinical (%)**	*Environmental (%)†*
L. pneumophila SG1	159 (64·1)	62 (92·5)	97 (53·6)
L. pneumophila SG3	17 (6.8)	0	17 (9·4)
L. pneumophila SG6	15 (6·0)	2 (2·9)	13 (7·2)
L. pneumophila SG7	1 (0·4)	0	1 (0·5)
L. pneumophila SG8	52 (20·9)	2 (2·9)	50 (27·6)
L. pneumophila SG8/10	3 (1·2)	0	3 (1·6)
L. bozemanii SG1	1 (0·4)	1 (1·5)	0
Total	248	67	181

* % refers to the total number of clinical isolates.
† % refers to the total number of environmental isolates.

Table 3. *Subgrouping of 158* L. pneumophila *SG1 isolates*

Major subgroup	Minor subgroup	Total (%)	Clinical isolates (%)	Environmental isolates (%)
Pontiac	Philadelphia 1 or Allentown 1	73 (55·3)*	24 (42·8)	49 (64·4)
	Benidorm 030E	29 (21·9)	19 (33·9)	10 (13·1)
	Knoxville 1	27 (20·4)	11 (19·6)	16 (21)
	France 5811	3 (2·2)	2 (3·5)	1 (1·3)
	Total	132 (83)	56 (90·3)	76 (78·3)
Olda	Olda	7 (31·8)	3 (50)	4 (25)
	Oxford 4032E or Camperdown 1	15 (68·1)	3 (50)	12 (75)
	Total	22 (13·8)	6 (9·6)	16 16·4)
Bellingham	Bellingham 1	5 (3·1)	0	5 (5·1)
Total		159	62	97

Note: Subgrouped using the following monoclonals: MAB1, MAB2, MAB3, 33G2, 144C and W268.
* % referred to isolates belonging to each major subgroup.

isolates (13 clinical, 28 environmental) belonged to Philadelphia 1 or Allentown 1 subtype. In Barcelona 17 of 36 isolates (31 clinical, 5 environmental) were Benidorm 030E, 13 were Philadelphia 1 or Allentown 1 (the remaining 6 isolates belonging to Knoxville 1 (2), Bellingham 1 (2), France 5811 (1) and Oxford 4032E or Camperdown 1(1)). In Valencia 15 of 17 isolates were Knoxville 1 and two were Philadelphia 1 or Allentown 1.

An analysis of subtyping samples from four outbreaks in which clinical and/or environmental isolates had been recovered is presented in Table 4. In three, the Philadelphia 1 or Allentown 1 subtype was the causative agent; in the fourth, the Knoxville 1 subtype was found.

DISCUSSION

The isolates included in this study form a heterogeneous group typical of the material submitted to a reference laboratory in which both diagnostic and reference services are performed. However, the wide geographical distribution of

Table 4. *Monoclonal typing of* L. pneumophila *SG1 isolates related to outbreaks*

Outbreak	Number	Origin	Number	Subtype
A	2	Clinical	2	Philadelphia 1 or Allentown 1
	11	Environmental	11	Philadelphia 1 or Allentown 1
B	8	Environmental	8	Philadelphia 1 or Allentown 1
C	1	Clinical	1	Knoxville 1
	14	Environmental	14	Knoxville 1
D	5	Clinical	5	Philadelphia 1 or Allentown 1
	6	Environmental	6	Philadelphia 1 or Allentown 1

isolates throughout 21 different provinces in Spain reinforces the representative nature of the studied sample. Moreover, all clinical isolates can be considered as pathogens, except for one *L. bozemanii* SG1 strain recovered from a catheter without clear evidence of having caused disease. Environmental isolates were recovered in most cases from case-associated buildings, although very frequently their involvement as an aetiological agent of disease could not be determined.

As expected, *L. pneumophila* SG1 was the most common serogroup, both in clinical and environmental isolates, followed by SG8, SG6 and SG3. The high proportion of SG8 isolates is noticeable, especially in environmental samples from two different localities, Alicante and Baleares; in Barcelona two isolates of this serogroup were recovered as pathogenic agents. There were three isolates labelled as SG8/10 which we were unable to allocate in an individual serogroup.

The high incidence of subgroup Pontiac in both clinical and environmental isolates correlates with the findings of other authors [11], confirming previous reports that the MAB-2 reacting antigen marker seems more often associated with virulence than antigens present in the other subgroups [12, 13]. Minor subgroup Philadelphia 1 or Allentown 1 was commonest in both environmental and clinical isolates (more than half of the strains) and was responsible for three of the outbreaks in which several isolates could be studied. This has also been described by others [6, 11].

Subgroups Olda and Bellingham were also present in a small number of clinical samples and in environmental ones, as in other studies [6, 11].

These findings support the applicability of monoclonal typing in the epidemiological studies of legionellosis especially in cases associated with buildings in which the source of infection is sought for control purposes. In these cases a bigger effort should be made to obtain representative isolates both from clinical cases and from water or other environmental sites which may be the origin of the infection.

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